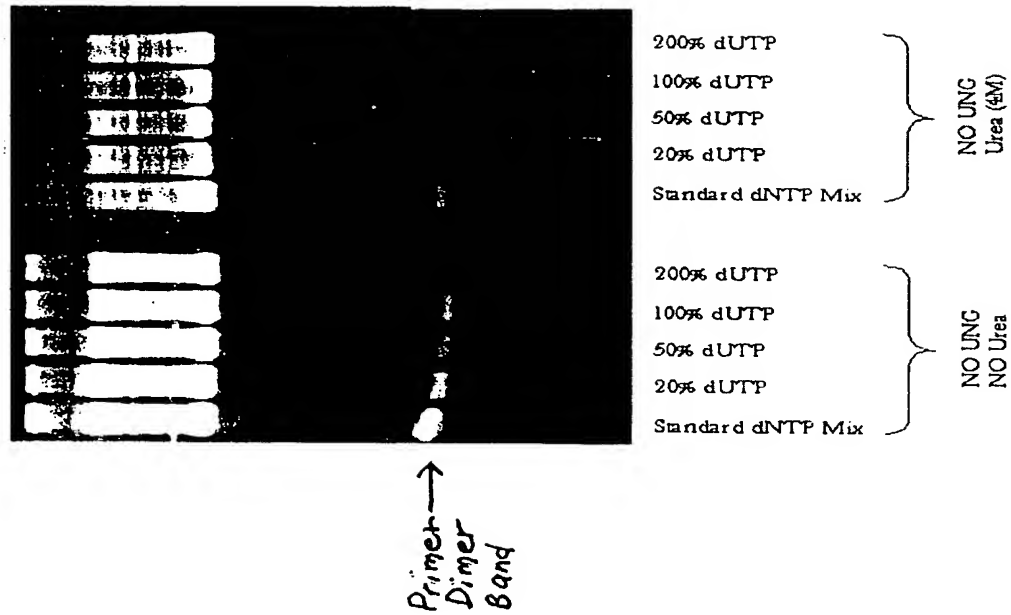
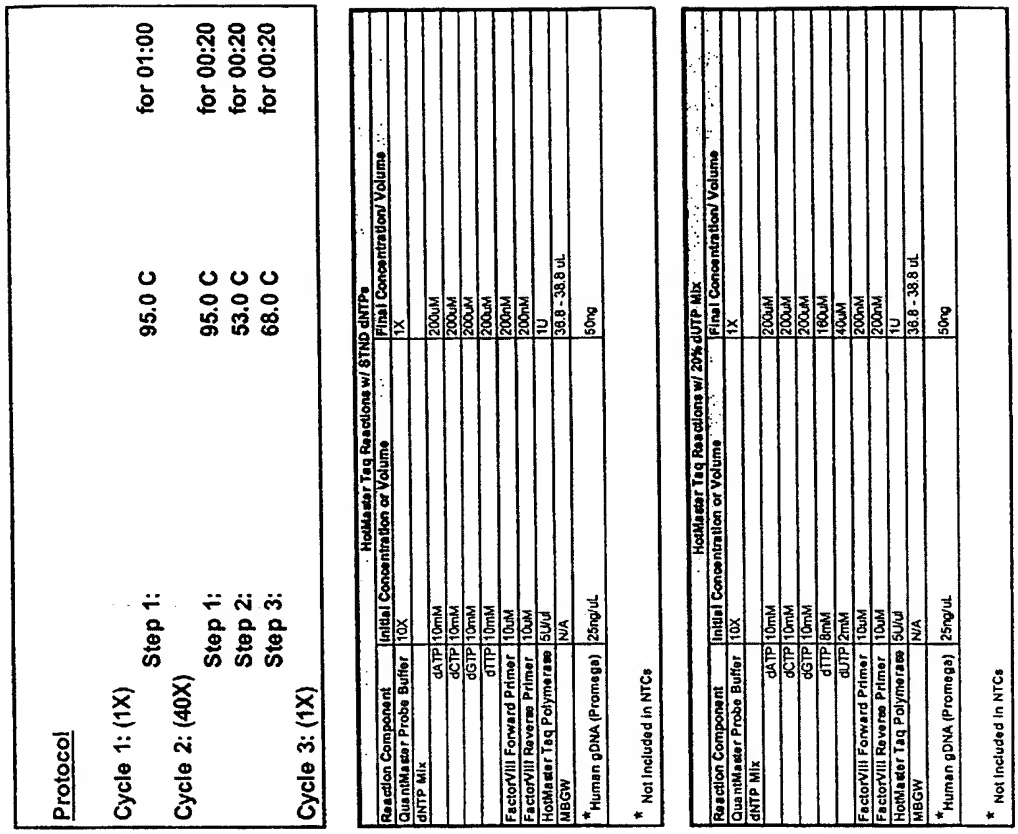
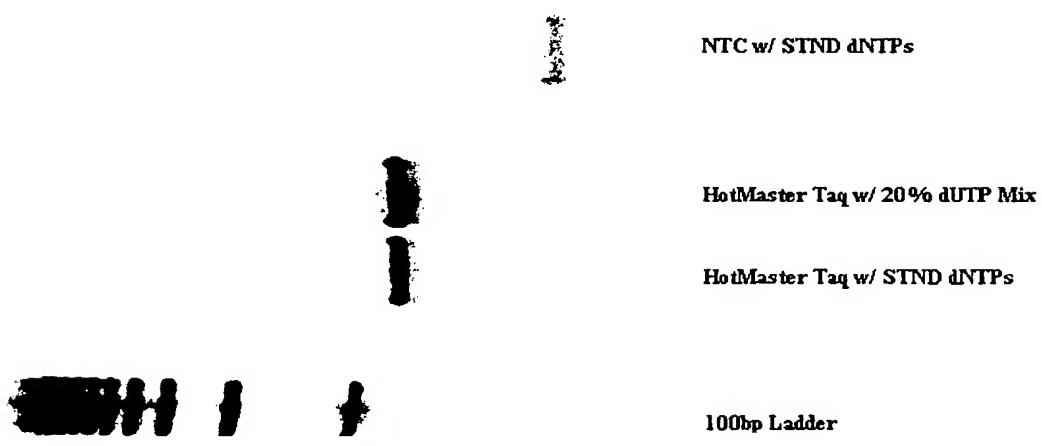
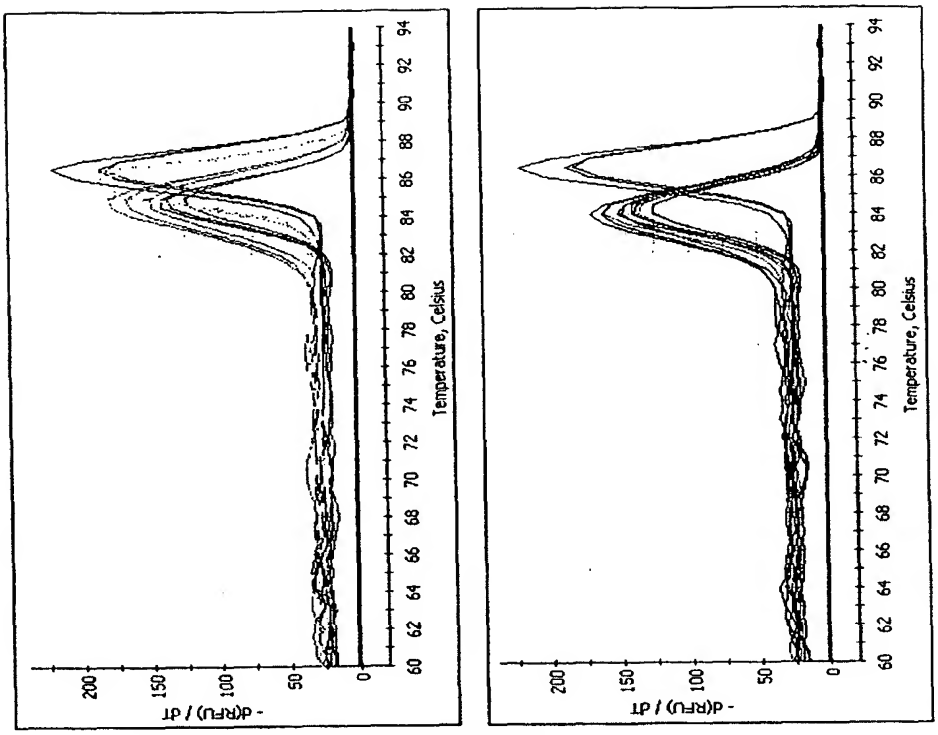

200% dUTP
100% dUTP
50% dUTP
20% dUTP
Standard dNTP Mix
NO UNG
Urea (4M)
200% dUTP
100% dUTP
50% dUTP
20% dUTP
Standard dNTP Mix
NO UNG
NO Urea
Primer Dimer Band

FIG. 1

FIG. 2


Protocol
Cycle 1: (1X)
Step 1: 95.0 C for 01:00
Cycle 2: (40X)
Step 1: 95.0 C for 00:20
Step 2: 53.0 C for 00:20
Step 3: 68.0 C for 00:20
Cycle 3: (1X)
HotMaster Taq Reactions w/ STND dNTPs
Reaction Component | Initial Concentration or Volume | Final Concentration/ Volume
QuantiMaster Probe Buffer | 10X | 1X
dNTP Mix
dATP | 10mM | 200uM
dCTP | 10mM | 200uM
dGTP | 10mM | 200uM
dTTP | 10mM | 200uM
FactorVIII Forward Primer | 10uM | 200nM
FactorVIII Reverse Primer | 10uM | 200nM
HotMaster Taq Polymerase | 5U/ul | 1U
MBGW | N/A | 36.8 - 38.8 uL
*Human gDNA (Promega) | 25ng/uL | 50ng
* Not Included in NTCs
HotMaster Taq Reactions w/ 20% dUTP Mix
Reaction Component | Initial Concentration or Volume | Final Concentration/ Volume
QuantiMaster Probe Buffer | 10X | 1X
dNTP Mix
dATP | 10mM | 200uM
dCTP | 10mM | 200uM
dGTP | 10mM | 200uM
dTTP | 8mM | 160uM
dUTP | 2mM | 40uM
FactorVIII Forward Primer | 10uM | 200nM
FactorVIII Reverse Primer | 10uM | 200nM
HotMaster Taq Polymerase | 5U/ul | 1U
MBGW | N/A | 36.8 - 38.8 uL
*Human gDNA (Promega) | 25ng/uL | 50ng
* Not Included in NTCs


NTC w/ 20% dUTP Mix
NTC w/ STND dNTPs
HotMaster Taq w/ 20% dUTP Mix
HotMaster Taq w/ STND dNTPs
100bp Ladder


- d(RFU) / dT
Temperature, Celsius
- d(RFU) / dT
Temperature, Celsius

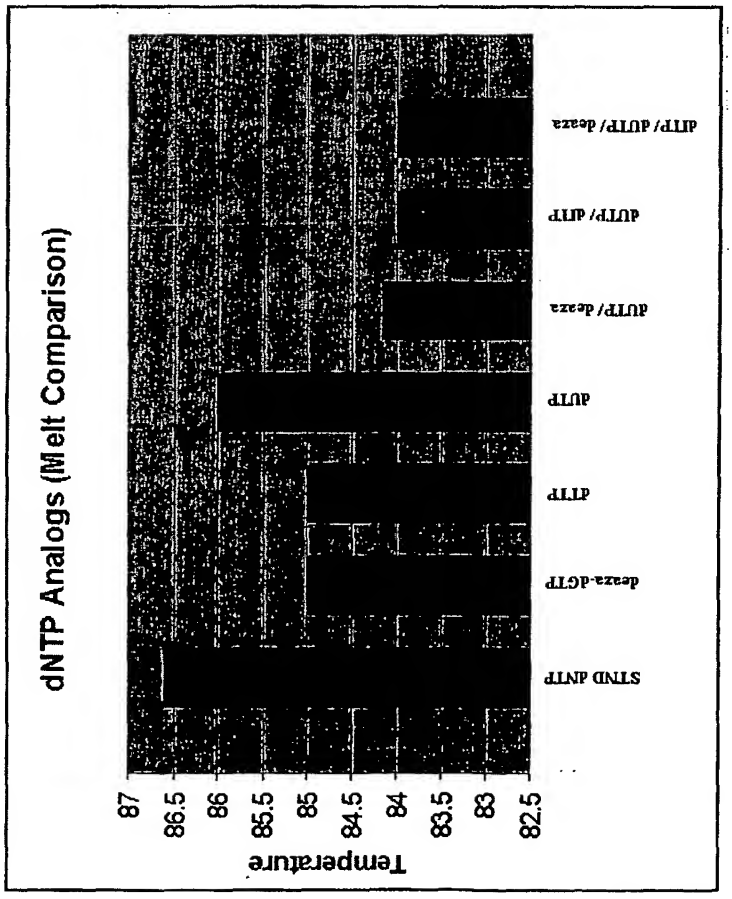

dNTP Analogs (Melt Comparison)
Temperature
87
86.5
86
85.5
85
84.5
84
83.5
83
82.5
STND dNTP
deaza-dGTP
dTTP
dUTP
dUTP/ deaza
dUTP/ dTTP
dTTP/ dUTP/ deaza

FIG. 3

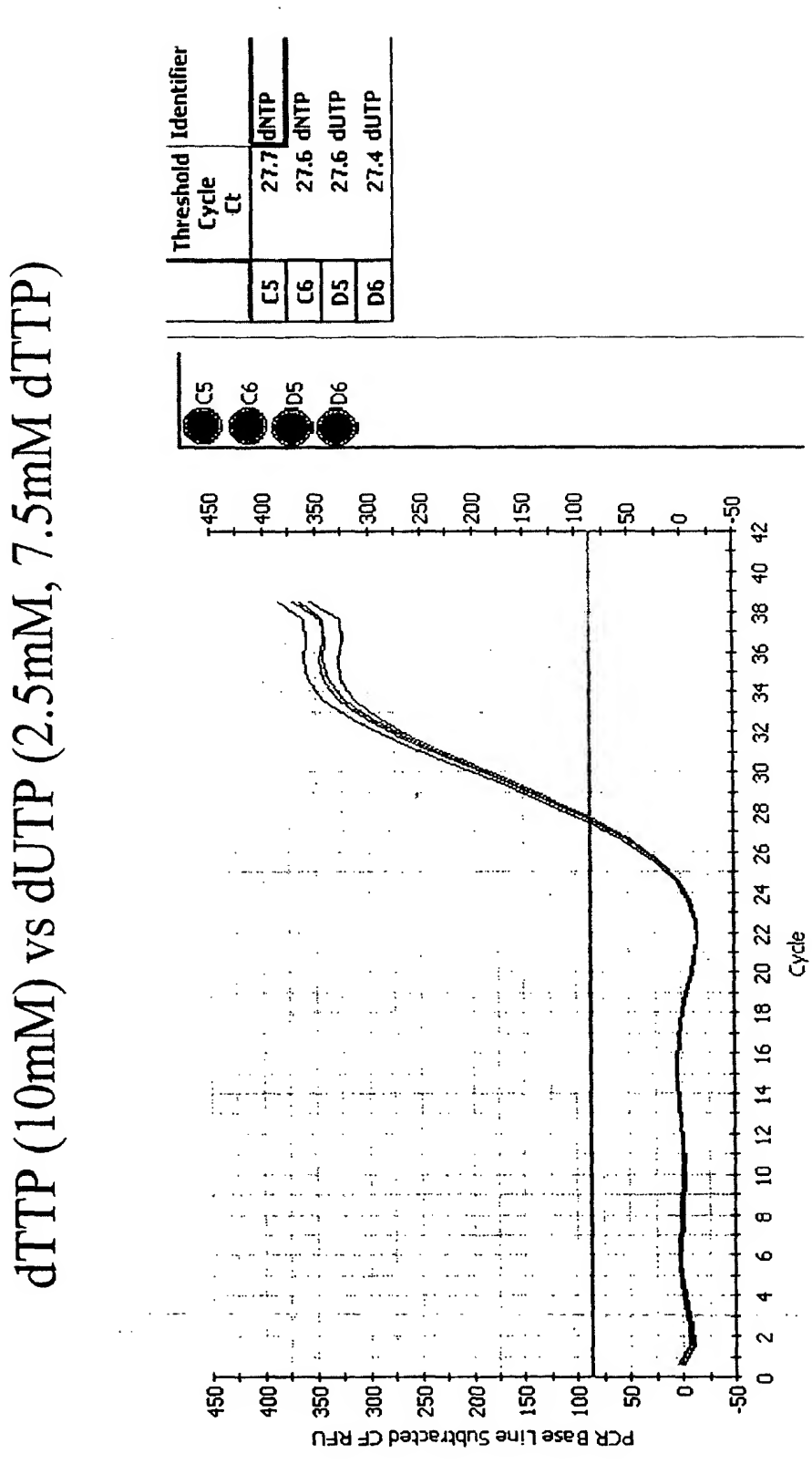


FIG. 4A

In real-time RT-PCR, the use of dTTP or a combination of dTTP and dUTP does not seem to affect the Ct or RFU significantly.

Standard dNTP mix with dTTP (10mM) vs dUTP mix (2.5mM dUTP, 7.5mM dTTP)

These results show that the addition of dUTP to the dNTP mix does not significantly affect the product yield.

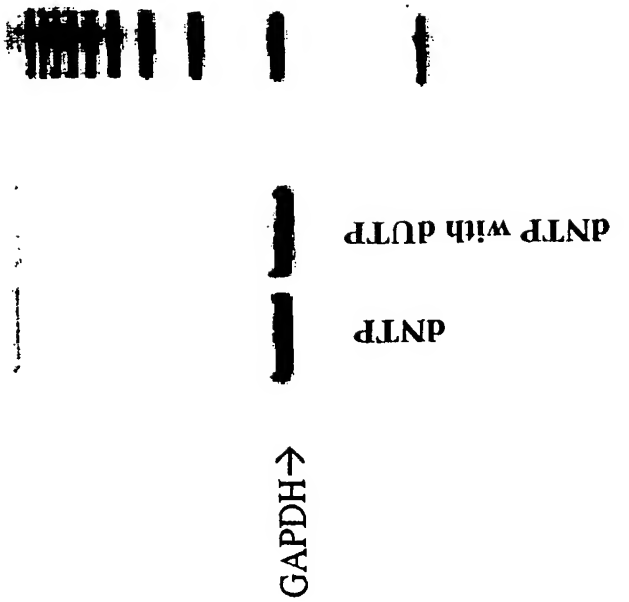


FIG. 4B

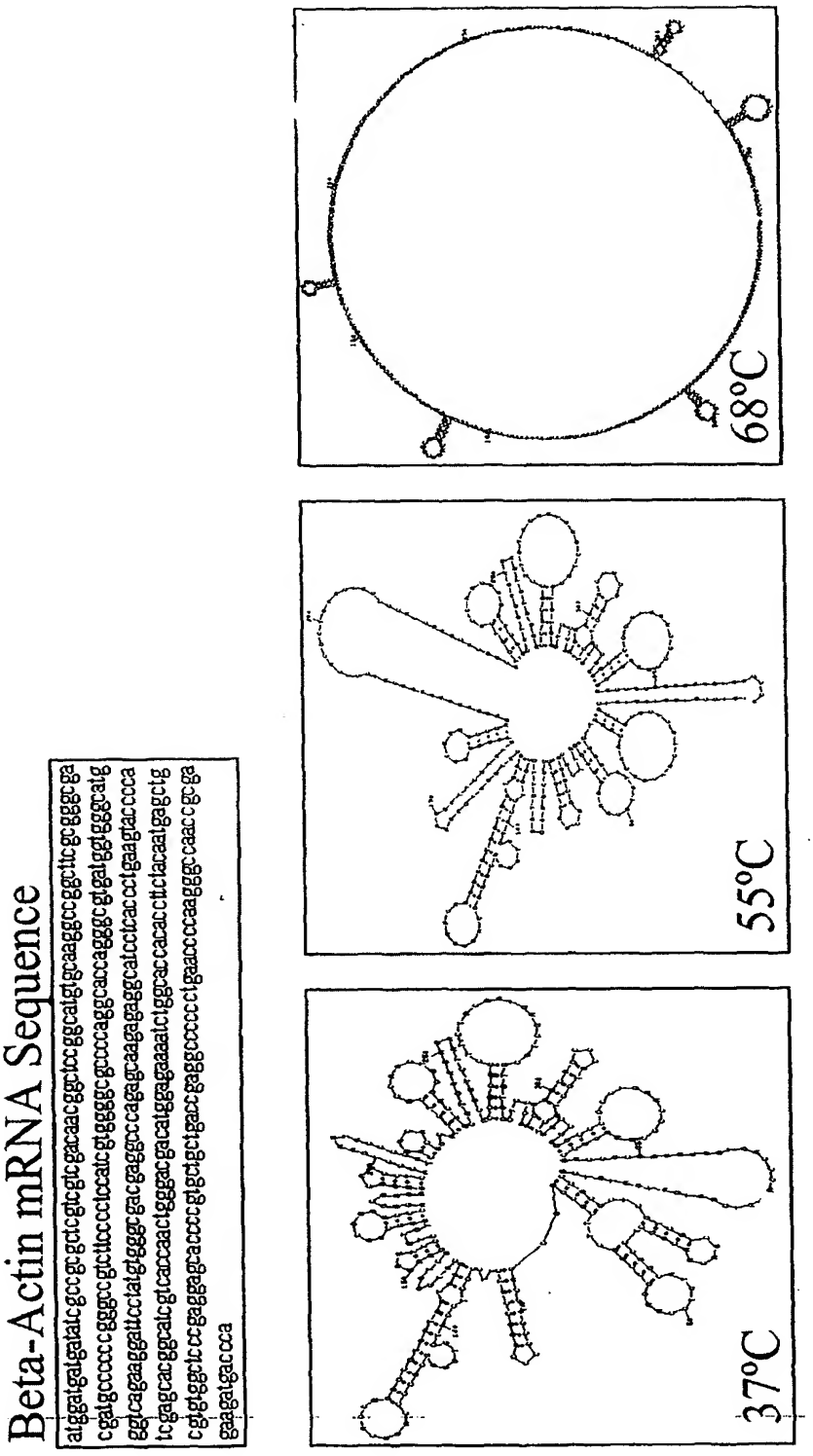

Beta-Actin mRNA Sequence
atggatgatgatatcgccgcgctcgtcgtcgacaacggctccggcatgtgcaaggccggcttcgcgggcgacgatgccccccgggccgtctttcccctccatcgtggggcgccccaggcaccagggcgtgatggtgggcatgggtcagaaggattcctatgtgggcgacgaggcccagagcaagagaggcatcctcaccctgaagtaccccatcgagcacggcatcgtcaccaactgggacgacatggagaaaatctggcaccacaccttctacaatgagctgcgtgtggctcccgaggagcacccgtgctgctgaccgaggccccctgaacccccaaagggccaaccgcgagaagatgaccca
68°C
55°C
37°C

FIG. 5A

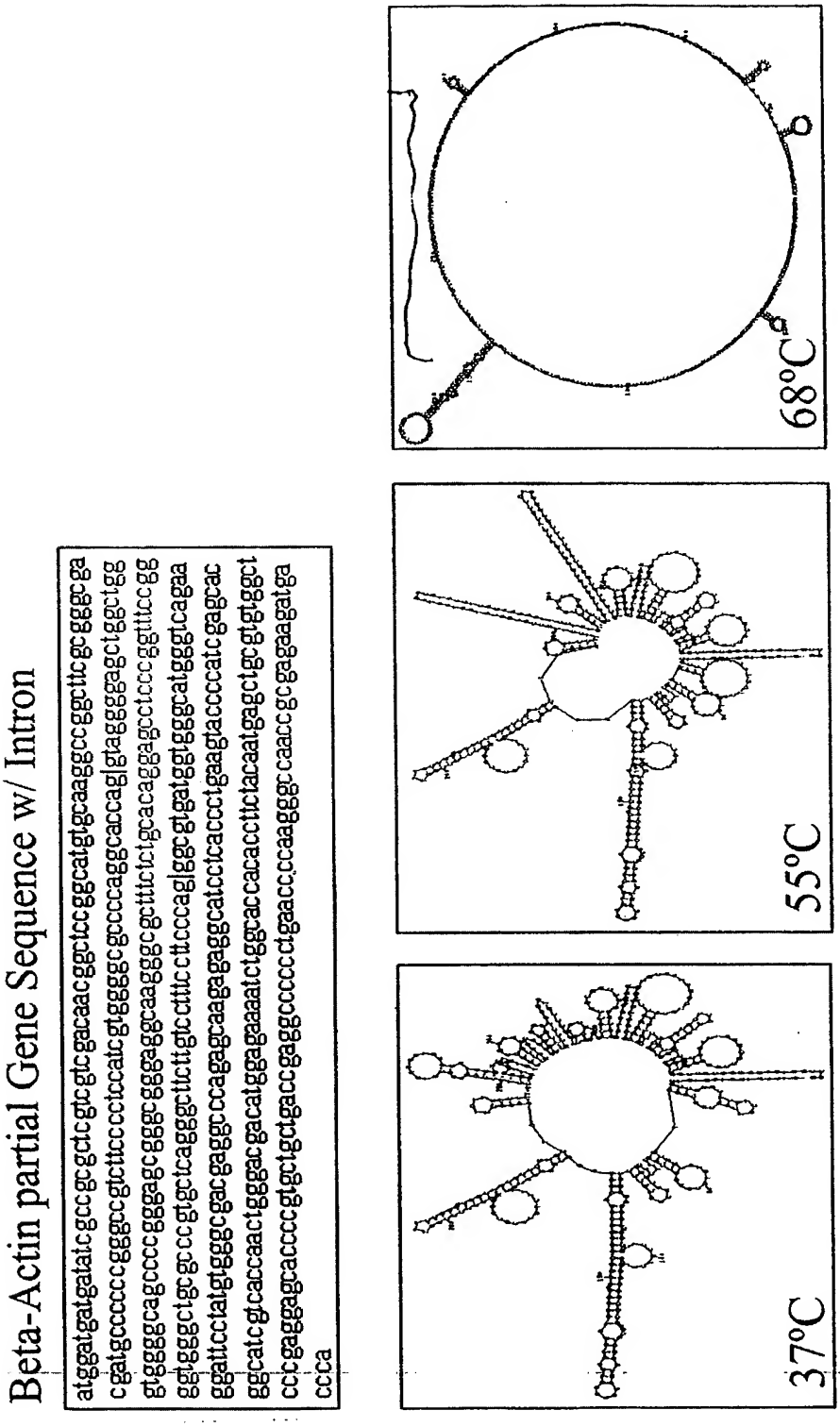

Beta-Actin partial Gene Sequence w/ Intron
atggatgatgatatcgccgcgctcgtcgtcgacaacggctccggcatgtgcaaggccggttccgcggggga
cgatgccccccgggggccgtcttccccttccatcgtgggggcgccccaggcaccaggtgatggggagctggctgg
gtgggggcagccccgggagcgggcgggaggcaagggcgctttctctgcacaggagcctcccgattccgg
ggtgggctgcgccgtcgtcagggcttcttgtcctttccccagtggcgtgatggtgggcatgggtcagaa
ggatcctatgtgggcgacgaggccaggagcaagagaggcatcctcaccctgaagtaccccatcgagcac
ggcatcgtcaccaactgggacgacatggagaaaatctggcaccacaccttctacaatgagctgcgtgtggct
cccgaggagcaccccgtgctgctgaccgaggccccctgaaccccaagggccaaccgcgagaagatga
ccca
37°C
55°C
68°C

FIG. 5B

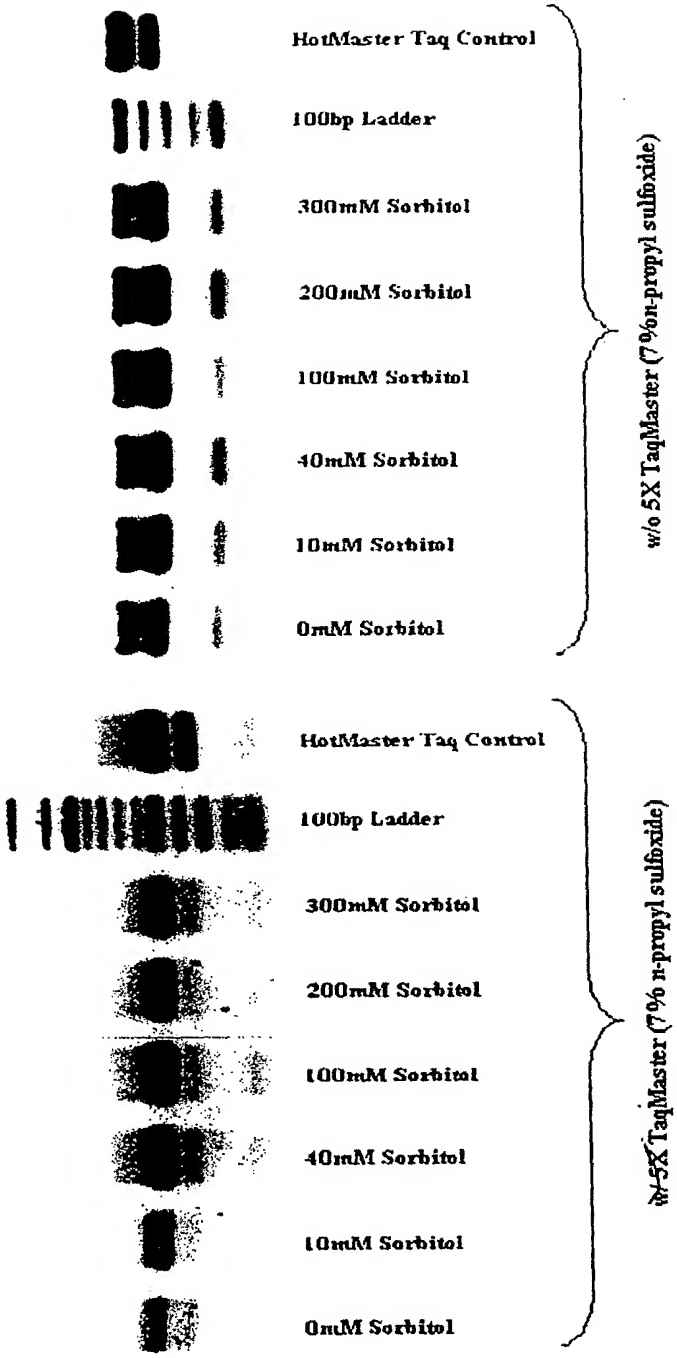
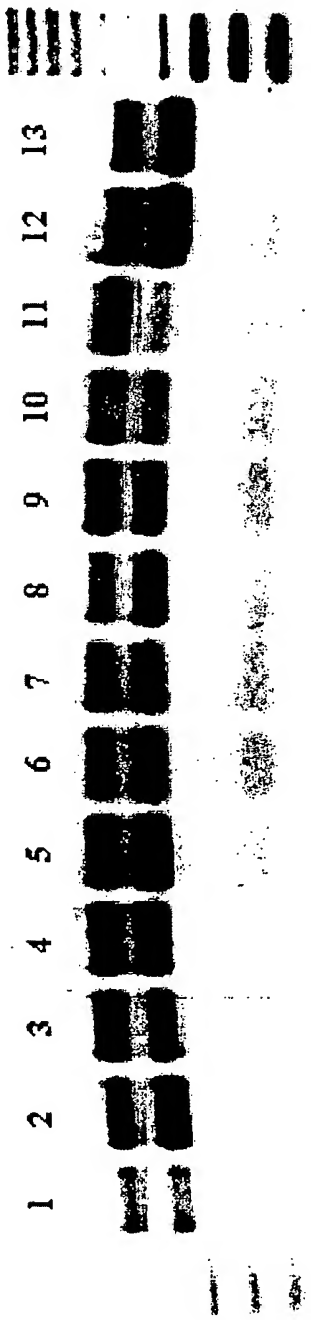

HotMaster Taq Control
100bp Ladder
300mM Sorbitol
200mM Sorbitol
100mM Sorbitol
40mM Sorbitol
10mM Sorbitol
0mM Sorbitol
w/o 5X TaqMaster (7%n-propyl sulfoxide)
HotMaster Taq Control
100bp Ladder
300mM Sorbitol
200mM Sorbitol
100mM Sorbitol
40mM Sorbitol
10mM Sorbitol
0mM Sorbitol
w/ 5X TaqMaster (7% n-propyl sulfoxide)

FIG. 6



100bp Ladder

25% dUTP HotMaster Control

STND dNTP HotMaster Control

25% dUTP/ 3%DMSO/ 300mM Mann/ SSBP

25% dUTP/ 3%DMSO/ 300mM Sorb/SSBP

25% dUTP/ 300mM Mannitol/ SSBP

25% dUTP/ 300mM Sorbitol/ SSBP

25% dUTP/ 3% DMSO/ SSBP

25% dUTP Mix/ 300mM Mannitol

25% dUTP Mix/ 300mM Sorbitol

25% dUTP Mix/ 3% DMSO

STND dTNP/ 300mM Mannitol

STND dNTP/ 300mM Sorbitol

STND dNTP/ 3% DMSO

100bp Ladder

FIG. 7A

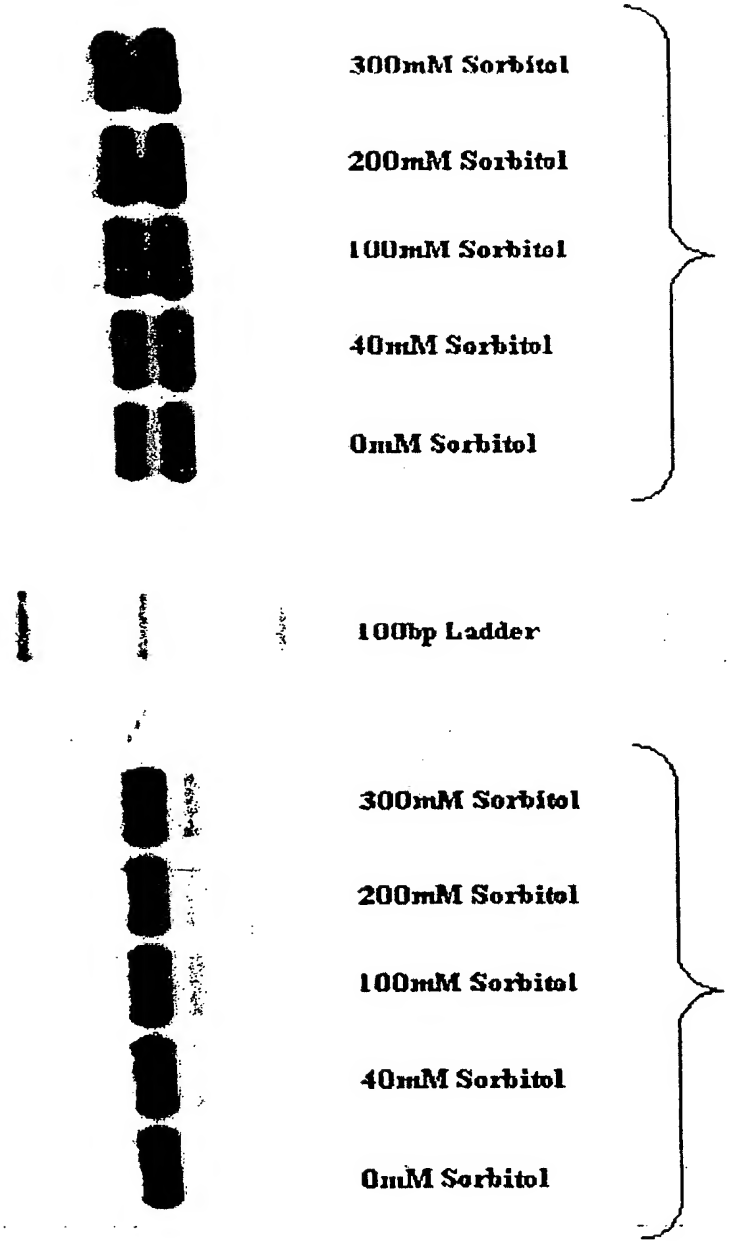

300mM Sorbitol
200mM Sorbitol
100mM Sorbitol
40mM Sorbitol
0mM Sorbitol
w/o 5X TaqMaster
(30mM Trehalose)
100bp Ladder
300mM Sorbitol
200mM Sorbitol
100mM Sorbitol
40mM Sorbitol
0mM Sorbitol
w/ 5X TaqMaster
(Trehalose 30mM)

FIG. 7B

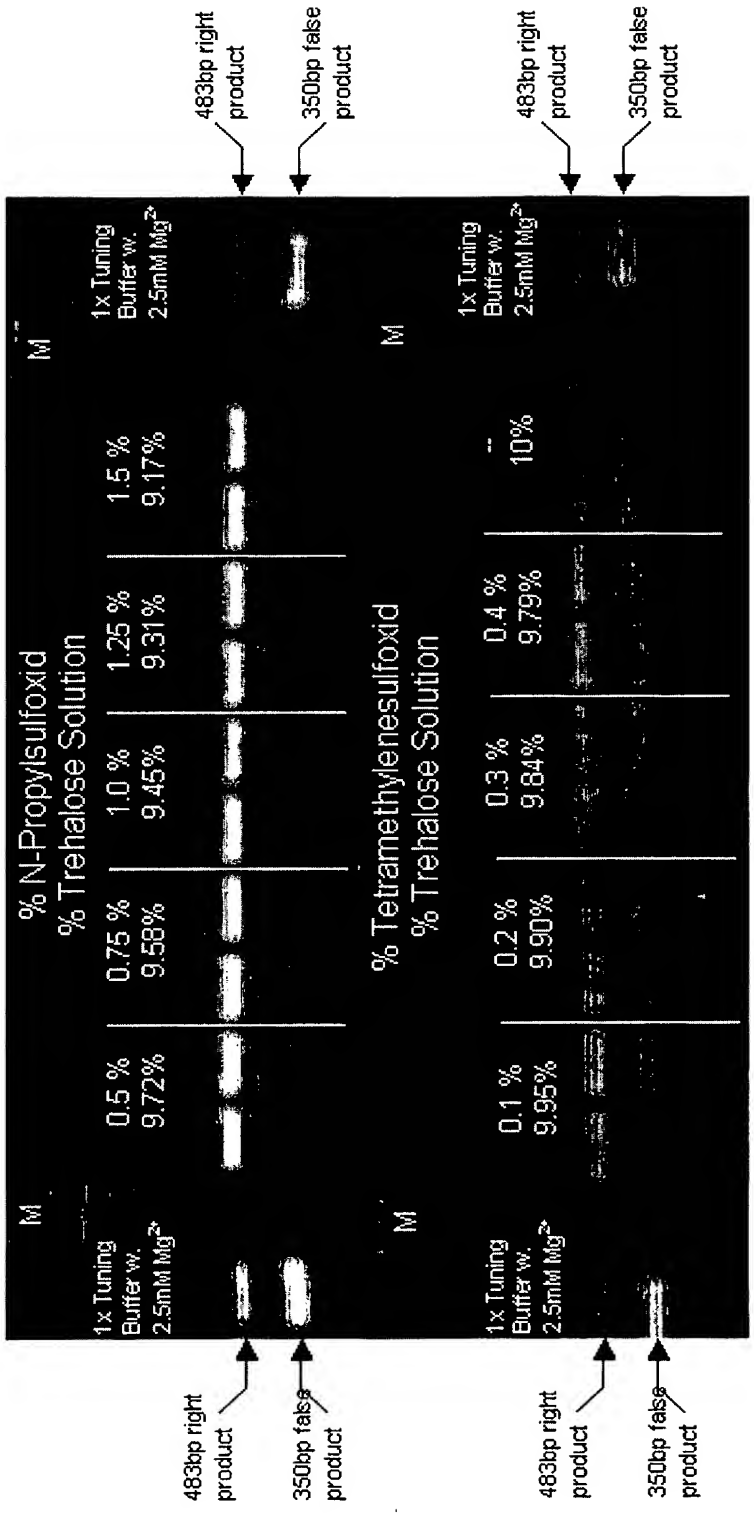

483bp right product
350bp false product
M
1x Tuning Buffer w. 2.5mM Mg2+
0.5 % 9.72%
0.75 % 9.58%
1.0 % 9.45%
1.25 % 9.31%
1.5 % 9.17%
% N-Propylsulfoxid % Trehalose Solution
0.1 % 9.95%
0.2 % 9.90%
0.3 % 9.84%
0.4 % 9.79%
-- 10%
% Tetramethylenesulfoxid % Trehalose Solution

FIG. 8

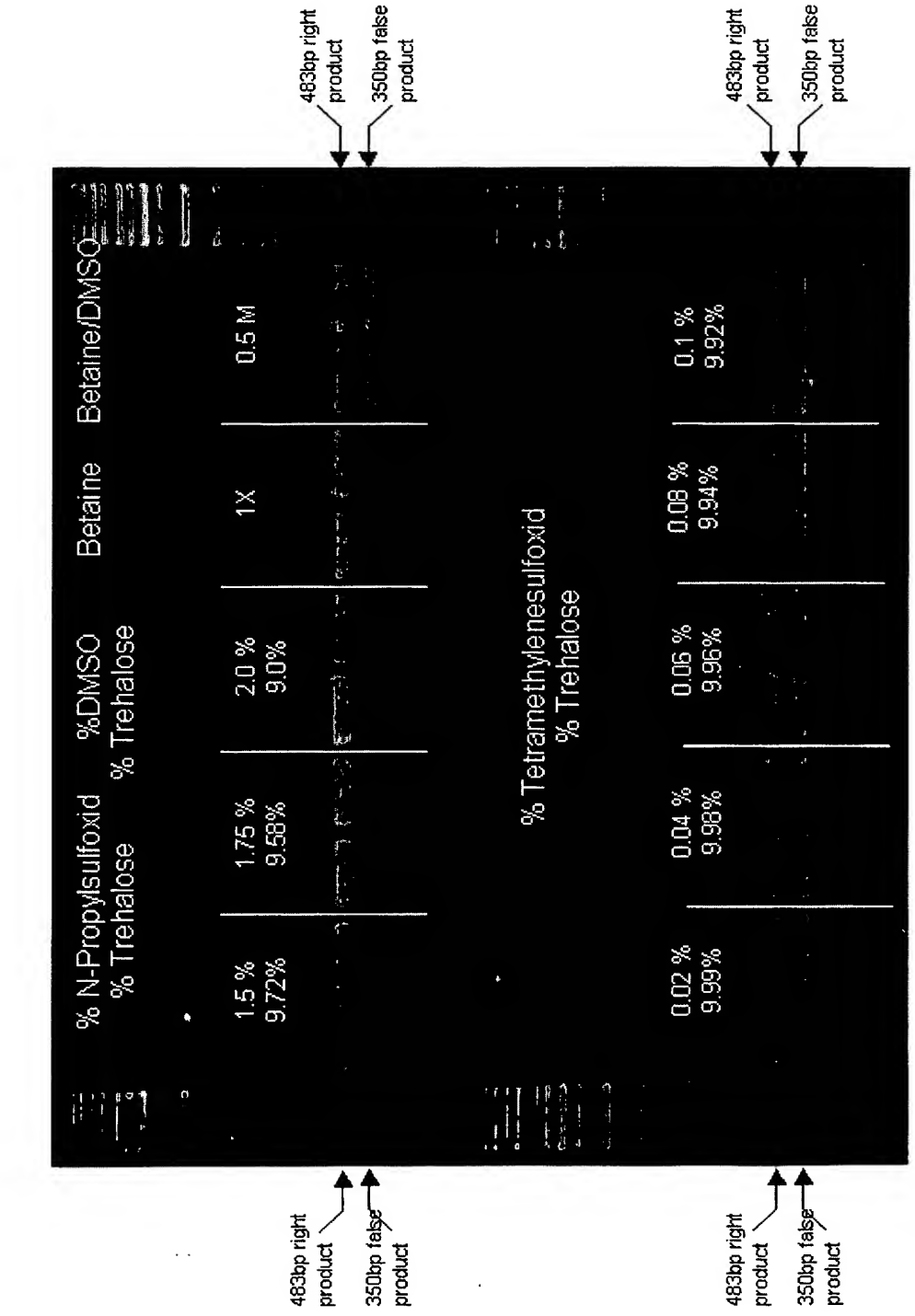

483bp right product
350bp false product
% N-Propylsulfoxid
% Trehalose
%DMSO
% Trehalose
Betaine
Betaine/DMSO
1.5 %
9.72%
1.75 %
9.58%
2.0 %
9.0%
1X
0.5 M
% Tetramethylenesulfoxid
% Trehalose
0.02 %
9.99%
0.04 %
9.98%
0.06 %
9.96%
0.08 %
9.94%
0.1 %
9.92%

FIG. 9